

CLAIMS

1. A substantially pure or isolated polypeptide which
 - a) consists of the amino acid sequence as shown in SEQ ID NO: 88, or
 - b) consists essentially of the amino acid sequence shown in SEQ ID NO: 88, and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex, or
 - c) consists essentially of an amino acid sequence with a sequence identity of at least 80% with SEQ ID NO: 88, and which is at least 6 contiguous amino acid residues of SEQ ID NO: 88, and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex; wherein "sequence identity" is a measure of the degree of similarity between two amino acid sequences of equal length calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences.
2. A substantially pure or isolated polypeptide which consists essentially of:
 - a) at least 6 contiguous amino acid residues of SEQ ID NO: 88, or
 - b) an amino acid sequence with a sequence identity of at least 80% with SEQ ID NO: 88, or
 - c) an amino acid sequence with a sequence identity of at least 80% with a); wherein the polypeptide is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex; wherein "sequence identity" is a measure of the degree of similarity between two amino acid sequences of equal length calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences.
3. A substantially pure or isolated polypeptide which consists of the amino acid sequence as shown in SEQ ID NO: 88.

4. A substantially pure or isolated polypeptide which consists essentially of the amino acid sequence as shown in SEQ ID NO: 88.
5. A substantially pure or isolated polypeptide which consists essentially of an amino acid sequence with a sequence identity of at least 80% with SEQ ID NO: 88, and
- 5 which is at least 6 contiguous amino acid residues of SEQ ID NO: 88 wherein the polypeptide is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or
- 10 ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex; wherein "sequence identity" is a measure of the degree of similarity between two amino acid sequences of equal length calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences.
- 15 6. A substantially pure or isolated polypeptide which consists essentially of at least 6 contiguous amino acid residues of SEQ ID NO: 88, wherein the polypeptide is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of
- 20 eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex.
7. The polypeptide of claim 1 or 2 consisting essentially of a T cell epitope of SEQ ID NO: 88 that is a non-naturally occurring polypeptide that induces a release of IFN- γ
- 25 from primed memory T-lymphocytes withdrawn from a mouse within 2 weeks of primary infection or within 4 days after the mouse has been re-challenge infected with mycobacteria belonging to the tuberculosis complex, the induction performed by the addition of the polypeptide of a suspension comprising about 200,000 spleen cells per ml, the addition of the polypeptide resulting in a concentration of 1-4 μ g polypeptide per ml
- 30 suspension, the release of IFN- γ being assessable by determination of IFN- γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension, and elicits a delayed type hypersensitivity reaction.
8. A substantially pure or isolated polypeptide which consists essentially of an amino acid sequence with a sequence identity of at least 80% with SEQ ID NO: 88, or
- 35 wherein the polypeptide is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the
- 40 tuberculosis complex; wherein "sequence identity" is a measure of the degree of similarity between two amino acid sequences of equal length calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences.

9. A substantially pure or isolated polypeptide which consists essentially of a first amino acid sequence with a sequence identity of at least 80% with a second amino acid sequence that consists essentially of at least 6 contiguous amino acid residues of SEQ ID NO: 88, wherein the polypeptide is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex; wherein "sequence identity" is a measure of the degree of similarity between two amino acid sequences of equal length calculated as $(N_{ref} - N_{dif}) * 100/N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences.

10. The polypeptide according to any one of claims 1 or 2 in essentially pure form.

11. The polypeptide according to any one of claims 1 or 2 which consists essentially of an epitope for a T-helper cell.

12. A substantially pure or isolated polypeptide which consists essentially of at least 7 contiguous amino acid residues of SEQ ID NO: 88 and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex.

13. A substantially pure or isolated polypeptide which consists essentially of at least 12 contiguous amino acid residues of SEQ ID NO: 88 and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex.

14. A substantially pure or isolated polypeptide which consists essentially of at least 20 contiguous amino acid residues of SEQ ID NO: 88 and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex.

15. A substantially pure or isolated polypeptide which consists essentially of at least 30 contiguous amino acid residues of SEQ ID NO: 88 and is immunologically equivalent to the amino acid sequence shown in SEQ ID NO: 88 with respect to the ability of evoking a protective immune response in mice against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex.

16. The polypeptide according to any one of claims 1 or 2 which is free from any signal sequence.

17. The polypeptide according to any one of claims 1 or 2 which

5 a) induces a release of IFN- γ from primed memory T-lymphocytes withdrawn from a mouse within 2 weeks of primary infection or within 4 days after the mouse has been re-challenge infected with mycobacteria belonging to the tuberculosis complex, the induction performed by the addition of the polypeptide to a suspension comprising about 200,000 spleen cells per ml, the addition of the polypeptide resulting in a concentration of 1-4 μ g polypeptide per ml suspension, the release of IFN- γ being assessable by determination of IFN- γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension, and/or

10 b) induces a release of IFN- γ of at least 300 pg above background level from about 1000,000 human peripheral blood mononuclear cells per ml isolated from TB patients in the first phase of infection, or from healthy BCG vaccinated donors, or from healthy contacts to TB patients, the induction being performed by the addition of the polypeptide to a suspension comprising the about 1,000,000 PBMC per ml, the addition of the polypeptide resulting in a concentration of 1-4 μ g polypeptide per ml suspension, the release of IFN- γ being assessable by determination of IFN- γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension; and/or

15 c) induces an IFN- γ release from bovine PBMC derived from animals previously sensitized with mycobacteria belonging to the tuberculosis complex, said release being at least two times the release observed from bovine PBMC derived from animals not previously sensitized with mycobacteria belonging to the tuberculosis complex.

20 18. The polypeptide according to any one of claims 1 or 2, wherein the sequence identity is at least 85%.

25 30 19. The polypeptide according to claim 18, wherein the sequence identity is at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or at least 99.5%.

30 20. The polypeptide according to claim 18, wherein the sequence identity is at least 95%.

35 35 21. The polypeptide according to claim 20, wherein the sequence identity is at least 96%, or at least 97%, or at least 98%, or at least 99%, or at least 99.5%.

40 22. A fusion polypeptide comprising at least one polypeptide according to any of claims 1 or 2 and at least one fusion partner.

45 23. A fusion polypeptide, consisting essentially of at least one polypeptide according to any one of claims 1 or 2 and at least one fusion partner selected from the group consisting of ESAT-6, at least one T-cell epitope of ESAT-6, MPB64, at least one T-cell epitope of MPB64, MPT64 at least one T-cell epitope of MPT64, and MPB59 and at least one T-cell epitope of MPB59.

50 24. The polypeptide according to any one of claims 1 or 2 which is lipidated.

25. A composition comprising a polypeptide according to any one of claims 1 or 2 and pharmaceutically acceptable carrier, vehicle or adjuvant.
26. An immunological composition comprising a polypeptide according to any one of claims 1 or 2.
- 5 27. The immunological composition according to claim 26, further comprising an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.
28. The immunological composition according to claim 27, wherein the carrier is a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction; the vehicle is selected from the group consisting of a diluent and a suspending agent; and
- 10 the adjuvant is Freund's incomplete adjuvant.
29. An immunological composition comprising at least two different polypeptides according to any one of claims 1 or 2.
30. An immunological composition comprising 3-20 different polypeptides according to any one of claims 1 or 2.
- 15 31. A skin test reagent comprising the immunological composition of claim 26.
32. A composition for diagnosing tuberculosis in an animal, including a human being, comprising a polypeptide according to any one of claims 1 or 2 optionally in combination with a means for detection.
33. A fusion polypeptide comprising at least one polypeptide according to any one of claims 3 or 4 and at least one fusion partner.
- 20 34. A fusion polypeptide, consisting essentially of at least one polypeptide according to any one of claims 3 or 4 and at least one fusion partner selected from the group consisting of ESAT-6, at least one T-cell epitope of ESAT-6, MPB64, at least one T-cell epitope of MPB64, MPT64 at least one T-cell epitope of MPT64, and MPB59 and at least
- 25 one T-cell epitope of MPB59.
35. The polypeptide according to any one of claims 3 or 4 which is lipidated.
36. A composition comprising a polypeptide according to any one of claims 3 or 4 and pharmaceutically acceptable carrier, vehicle or adjuvant.
37. An immunological composition comprising a polypeptide according to any one of claims 3 or 4.
- 30 38. The immunological compositions according to claim 37, further comprising an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.
39. The immunological composition according to claim 38, wherein the carrier is a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction;
- 35 the vehicle is selected from the group consisting of a diluent and a suspending agent; and the adjuvant is Freund's incomplete adjuvant.
- 40 1 40. An immunological composition comprising at least two different polypeptides according to claim 4.
41. An immunological composition comprising 3-20 different polypeptides according
- 40 to claim 4.
42. A skin test reagent comprising the immunological composition of claim 37.
43. A composition for diagnosing tuberculosis in an animal, including a human being, comprising a polypeptide according to any one of claims 3 or 4 optionally in combination with a means for detection.

44. A diagnostic tool comprising a combination of two or more substantially pure polypeptides, of which one or more comprises one or more amino acid sequences selected from

- (a) SEQ ID NO: 88;
- 5 (b) an immunogenic portion of the sequence in (a); and /or
- (c) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.

45. A serodiagnostic composition comprising a combination of two or more substantially pure polypeptides, of which one or more comprises one or more amino acid sequences selected from

- (a) SEQ ID NO: 88;
- (b) an immunogenic portion of the sequence in (a); and /or
- 15 (c) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.

46. A composition according to claim 32 or claim 43, which further comprises one or more amino acid sequences selected from the group consisting of:

- d) an amino acid sequence selected from the sequences shown in SEQ ID NO: 20 2, 4, 6, 8, 10, 12, 14, 16, 17-23, 42, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72-86, 90, 92, 94, 141, 143, 145, 147, 149, 151, 153, and 168-171;
- e) an immunogenic portion of any one of the sequences in (d); and
- f) an amino acid sequence analogue having at least 70% sequence identity to 25 any one of the sequences in (d) or (e) and at the same time being immunogenic.

47. A fusion protein according to claim 22, comprising as a fusion partner a polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

- 30 a) an amino acid sequence selected from the sequences shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 17-23, 42, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72-86, 90, 92, 94, 141, 143, 145, 147, 149, 151, 153, and 168-171;
- b) a polypeptide fragment derived from a virulent mycobacterium, such as 35 ESAT-6, MPB64, MPT64, TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, Ag85A, Ag85B, Ag85C, 19kDa lipoprotein, MPT32, MPB59 and alpha-crystallin;
- c) an immunogenic portion of any one of the sequences in (a) or (b); and
- d) an amino acid sequence analogue having at least 70% sequence identity to 40 any one of the sequences in (a), (b), or (c) and at the same time being immunogenic.